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Effect of Carbon Tetrachloride
Treatment on Urine Flow Rate of the
Rainbow Trout 'Salmo gairdneri'

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SHORT COMMUNICATIONS

The Effect of Carbon Tetrachloride Treatment on Urine Flow Rate of the Rainbow Trout, *Salmo gairdneri*¹⁻³

The Effect of Carbon Tetrachloride Treatment on Urine Flow Rate of the Rainbow Trout, *Salmo gairdneri*. PFEIFER, K. F., AND WEBER, L. J. (1980). *Toxicol. Appl. Pharmacol.* 52, 347-350. Urine flow rate (UFR) of rainbow trout was measured as an assessment of water balance after treatment with a single ip dose of CCl₄ (2.0 ml/kg). CCl₄ produced an oliguria or anuria as early as 1 hr post-treatment and a significant reduction in the 24-hr urine output. UFR for control fish appeared to follow a diurnal pattern. Additionally, CCl₄-treated fish had a significant increase in relative wet body weight that was negatively correlated to UFR. The rainbow trout normally excretes a hypoosmotic urine; therefore, an increase in urine osmolality and a slight proteinuria further suggested that treated fish had suffered an alteration in renal homeostasis. Histological examination of kidney tissue at 24 hr post-treatment revealed early, localized pathological changes; however, extensive morphological damage was absent. The early oliguria and the lack of demonstrable renal histopathological changes in CCl₄-treated rainbow trout suggest that the reduction in UFR was not the result of a direct toxic effect on the kidney.

Carbon tetrachloride (CCl₄) is a hepatotoxic agent that has been widely used in experimental mammalian toxicology (Curtis *et al.*, 1972; Diaz Gómez *et al.*, 1975; Korsrud *et al.*, 1972; Lindstrom and Anders, 1977; Recknagel, 1967). Previous studies have established that rainbow trout, *Salmo gairdneri*, are susceptible to the hepatotoxic effects of CCl₄ (Gingerich *et al.*, 1978; Pfeifer *et al.*, 1977; Racicot *et al.*, 1975; Statham *et al.*, 1978). In addition to its deleterious action on liver structure and function, CCl₄ has been reported to be nephrotoxic to humans and laboratory mammals (Moon, 1950; Plaa and Larson, 1965; Sirota, 1949; Stricker *et al.*, 1968; Von Oettingen, 1955).

The effects of exogenous chemicals on salmonid renal function have not been ex-

tensively studied. No information is currently available on the nephrotoxic response of fish to CCl₄. The purpose of this study was to determine the effect of CCl₄ treatment on water balance in the rainbow trout by measuring urine flow rate (UFR).

METHODS

Rainbow trout (200-500 g) were purchased from Roaring River Hatchery, Scio, Oregon, and held in aquaria (200 gal) supplied with continuously flowing well water (15 ± 1.0°C). Animals were fed a commercial fish diet, Donaldson-Ore Aqua,⁴ daily until 24 hr prior to an experiment. A 12-hr light:dark photoperiod was used throughout all experiments. The study was conducted during the months of December through February.

Fish were initially anesthetized in a solution of tricaine methane sulfonate (MS-222, 50 mg/liter) for 3-5 min and weighed. During catheterization of the urinary bladder the desired level of anesthesia was maintained by irrigating the gills with MS-222 (100 mg/liter) or well water without anesthetic. Polyethylene tubing (PE-50) was inserted through the urogenital opening into the urinary bladder to a depth of 1.5-2.0 cm and sutured to the base of the anal fin.

Fish were placed in a Plexiglas restraining chamber which prevented vertical and lateral body movements

⁴ Oregon Aqua Foods, Newport, Ore.

¹ Oregon Agricultural Experiment Station Technical Paper No. 5095.

² A portion of this work was presented at the 176th National meeting of the American Chemical Society, September 1978, in Miami Beach, Fla.

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TABLE 1

MEAN URINE OUTPUT AND RELATIVE BODY WEIGHT CHANGE FOR NONTREATED RAINBOW TROUT DURING 48 hr AND FOR CORTLAND SALINE OR CCl₄-TREATED (2.0 ml/kg, ip) RAINBOW TROUT DURING 24-hr PRETREATMENT AND 24-hr POST-TREATMENT COLLECTION PERIODS

Parameter	Time (hr) ^a	
	1-24 ^b	25-48 ^b
Urine output (ml/kg/24 hr)		
Nontreated control (9)	103.2 ± 11.0 ^c	82.7 ± 12.0
Cortland control (8)	98.6 ± 6.7	104.1 ± 7.5
CCl ₄ (10)	98.7 ± 11.5	23.2 ± 10.1 ^d
Weight change (g/100 g body wt)		
Nontreated control	—	-2.8 ± 1.0 ^c
Cortland control	-2.3 ± 0.4	-0.3 ± 0.3
CCl ₄	-2.5 ± 0.4	3.7 ± 0.7 ^d

^a Hours postcatheterization.

^b Hours 1-24 and 25-48 represent the 24 hr pre- and post-treatment, respectively, for Cortland control and CCl₄-treated fish.

^c Values are mean ± SE for number of fish in parentheses.

^d Significantly different from Cortland control value ($p < 0.01$).

^e Weight change for 48 hr postcatheterization.

but permitted normal opercular respiration. The restraining chamber was located in a 57-liter aquaria and supplied with well water ($15 \pm 1.0^\circ\text{C}$) at 10 liters/min. Urine was collected at 1-hr intervals by gravity flow into test tubes in a fraction collector.

After collecting urine for 24 hr, fish were removed from the restraining chamber, anesthetized in MS-222 (50 mg/liter), weighed, and injected with undiluted CCl₄ (2.0 mg/kg, ip), at a point equidistant between the base of the pelvic fins and the distal edges of the pectoral fins. Control fish received an equivalent volume of Cortland physiological saline (Wolfe, 1963). Fish were returned to the chamber, and urine was collected for another 24-hr period. Additionally, urine was collected from nine fish for 48 hr without interruption, i.e., nontreated control fish. All fish were weighed at the end of the 48-hr experimental period.

Urine osmolality was determined with a Wescor³ vapor pressure osmometer every 12 hr postcatheterization. Approximate urine protein concentrations were obtained by a modified Albustix⁴ dip-stick method. A standard albumin solution was diluted to concentrations of 10, 20, 40, and 80 mg/100 ml, and the color from the urine sample was compared to the color of the standard. Urine was tested for protein every 6 hr during the 48-hr collection period.

Histological sections of the trunk kidney were obtained from fish that had not been catheterized but otherwise had been treated identically to the catheterized fish.

The kidney section was removed 24 hr post-treatment with CCl₄ (four fish) or Cortland saline (two fish) and fixed in Bouin's solution. Six-micrometer tissue sections were stained with hematoxylin and eosin and evaluated for morphological changes.

Experimental data were converted to a quantal form and analyzed by the Fisher exact probability test (Siegel, 1956). Values greater than 2 SD ($p < 0.05$) from the mean nontreated control value were chosen to indicate a positive response in the experimental fish. Levels of significant difference between Cortland control and CCl₄-treated fish were calculated by the method outlined in the Fisher test.

RESULTS AND DISCUSSION

The 24-hr mean urine output for CCl₄-treated fish decreased ($p < 0.01$) compared to the Cortland control (Table 1). Additionally, the post-treatment values for the CCl₄-treated fish were less ($p < 0.01$) than the pretreatment measurements. Oliguria or anuria in CCl₄-treated rainbow trout occurred as early as 1 hr after treatment and was still apparent at 24 hr. An apparent diurnal urine excretion pattern was evident for the Cortland controls during the 48-hr collection period and for the CCl₄-treated fish

³ Wescor, Inc., Logan, Utah.

⁴ Ames, Co., Elkhart, Ind.

during the 24-hr pretreatment period. The diurnal pattern of urine flow was completely abolished after treatment with CCl_4 .

The mean relative wet body weight for CCl_4 -treated fish compared to the Cortland controls increased ($p < 0.01$) during the 24-hr post-treatment period (Table 1). Relative wet body weight changes for Cortland control and nontreated control fish were similar for the 48-hr collection period. A negative correlation ($r = 0.80$) was established between urine output (ml/100 g/24 hr) and relative change in body weight (g/100 g/24 hr) for CCl_4 -treated fish during the 24-hr pre- and post-treatment periods.

Urine osmolality for the CCl_4 -treated fish decreased from 75 to 56 mosm/kg during the 24-hr pretreatment period but increased ($p < 0.01$) when compared to Cortland controls at the three test times during the 24-hr post-treatment period.

All urine samples from Cortland controls were negative for protein, i.e., < 10 mg/100 ml. At least 30% of CCl_4 -treated fish had a demonstrable proteinuria at some time post-treatment. The proteinuria varied considerably among individual fish at the same test time.

Histopathological evaluation of kidney tissue from CCl_4 -treated fish revealed no extensive morphological damage.⁷ Subtle changes, such as alteration in the brush border of the first and second proximal tubule segments, and localized hematopoietic cell necrosis occurred in one fish. No damage to glomeruli, distal tubule segments, or collecting ducts was noted in any kidney sections from CCl_4 -treated fish.

The kidney of the rainbow trout functions primarily as a water excretory organ. The urine of these fish is characteristically hypoosmotic to the plasma (Fromm, 1963; Hunn and Willford, 1970). The UFR is directly proportional to the glomerular fil-

tration rate (GFR) (Hickman, 1965). A nearly constant proportion of water and salts is reabsorbed by the renal tubules; therefore, variation in UFR is achieved by changes in GFR. In relatively resting conditions, the rainbow trout does not drink fresh water (Shehadeh and Gordon, 1969); therefore, the net volume of urine excreted should balance the net quantity of water entering through the gills.

Previous studies with salmonid fishes demonstrated that the stress of handling, exposure to sublethal concentrations of chemicals or hypoxic conditions can increase UFR (Hunn, 1969; Hunn and Allen, 1975; Lloyd and Orr, 1969; Swift and Lloyd, 1974). Lloyd and Orr (1969) attributed the diuretic response of rainbow trout exposed to sublethal concentrations of ammonia to an increase in gill permeability to water, rather than to a direct action on the kidney. It is unlikely that the decrease in UFR in our trout treated with CCl_4 was caused by a reduced water influx at the gills. These fish had a significant weight gain that was correlated to a decrease in UFR resulting from the inability to remove body water. Gingerich *et al.* (1978) reported a similar gain in body weight for rainbow trout at 24 hr post-treatment with CCl_4 .

The oliguria produced by CCl_4 treatment in our trout cannot be explained by renal histopathology. An increase in urine osmolality and slight proteinuria in CCl_4 -treated animals suggested a direct nephrotoxic effect; however, no glomerular damage or proximal tubular necrosis was observed in the fish at 24 hr post-treatment.

The present study demonstrated that UFR is an indicator of acute CCl_4 toxicity in rainbow trout and suggested that the early reduction in UFR was not caused by observable pathological changes in the kidney.

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⁷ Tissue sections were examined by Dr. Jerry Hendricks, Fish Pathologist, Department of Food Science, Oregon State University.

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